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GENE DOSAGE EFFECT FOR GLYCINAMIDE RIBONUCLEOTIDE SYNTHETASE IN HUMAN FIBROBLASTS TRISOMIC FOR CHROMOSOME 21

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Summary: The mean activity of glycinamide ribonucleotide synthetase of fibroblasts from fetuses with trisomy 21 is 1.64 - times that of normal cells. This gene dosage effect is confirmatory of the assignment of the gene for this enzyme to human chromosome 21.

Glycinamide ribonucleotide (GAR) synthetase (GARS; E.C.6.3.4.13), the third enzyme in the purine biosynthetic pathway (1), has been reported to be coded for by a gene on human chromosome 21 (2). This chromosome is of particular interest since, in the trisomic state, it is associated with Down syndrome, a common cause of mental retardation and abnormalities of development. The presence of an extra chromosome is generally accompanied by a commensurate gene dosage effect, with 50% increases in the activity of enzymes determined by that chromosome (3-5), and such a gene dosage effect has been demonstrated for soluble superoxide dismutase (SODs, SOD-1), another gene known to be carried by chromosome 21 (4). To determine whether such a dosage effect exists for GARS, and thereby to test the validity of the assignment of its gene to chromosome 21, we have compared the activities of GARS in trisomy 21 and normal fetal fibroblasts.

MATERIALS AND METHODS

Fibroblast Strains

Fibroblast strains were developed from the lung, skin, and/or diaphragm of 3 trisomic and 4 normal fetuses between 19 and 40 gestational weeks of age. Aside from a pair of dizygotic twins, one trisomic and the other normal, all donors were unrelated.

Preparation of Cellular Extracts

Cells were grown in Dulbecco modified Eagle medium (Gibco, Grand Island, N.Y.) containing 10% fetal calf serum, 100 u/ml penicillin and 100 µg/ml streptomycin in 5% CO_2 at 37°C. The cells were harvested when they reached 50 to 75% confluence, washed in saline G and in a buffer of 10 mM Tris-HCl,

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pH 7.3, 0.14 M NaCl, 5 mM $MgCl_2$, and 0.29 M sucrose, and lysed in 10 mM Tris-HCl, pH 7.3 with 0.29 M sucrose, as described by Oates (6). Protein concentrations were determined by the method of Lowry using bovine serum albumin as the standard.

Assays of Enzyme Activity

The assay for GARS was performed as described by Oates (6). [^{14}C]glycine (113 in Ci/mmole, New England Nuclear) was used as the radioactive substrate and was converted to the labile immediate substrate of the reaction, [^{14}C] phosphoribosylamine, by the activity of phosphoribosylpyrophosphate (PRPP) amidotransferase in the extract.

The assay for PRPP amidotransferase was performed as described by Martin (7). Dowex 1-X2 cleaned [^{14}C]L-glutamate (1.6 Ci/mmole, New England Nuclear) was used as the radioactive substrate. For both assays, the reaction products were spotted on DEAE paper discs, washed in de-ionized water, dried, and counted in a Beckman LS 8100 liquid scintillation spectrometer. The concentration of the original cell extracts was 0.8 ml protein/ml and the final concentration in the assay 0.4 mg/ml.

RESULTS

Three separate experiments, each with freshly prepared cell extracts, were performed, and the results are shown in Figure 1. In each experiment, the GARS activity in the trisomic cells was significantly greater than in normal cells, with $p = 0.025$, 0.005, and 0.05, respectively, by the one-tailed Wilcoxon rank order test. The individual ratios of trisomic/normal activities were 1.72, 1.66, and 1.53, respectively, with a mean of 1.64.

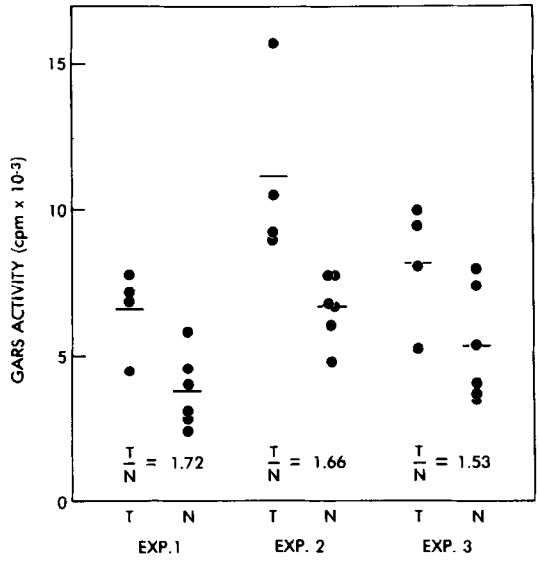

Figure 1 The activity of glycinamide ribonucleotide synthetase in extracts of trisomy 21 and normal human fetal fibroblasts. The results of three independent experiments are shown, and the mean ratio of trisomic to normal (T/N) activities was 1.64.

The generation of the immediate GARS substrate, phosphoribosylamine, requires the action of PRPP glutamine amidotransferase, Therefore, the activity of this enzyme in trisomic and normal fibroblast extracts was determined to insure that the observed differences in GARS activity did not actually represent a difference in PRPP amidotransferase. However, the activities of PRPP amidotransferase in the two types of extracts were not found to be significantly different.

DISCUSSION

The 1.64-fold increase in mean GARS activity in fibroblasts obtained from individuals with trisomy 21 is quite close to the expected gene dosage effect of 1.5. Spurious elevations of enzyme activities do not appear to occur in trisomy 21 fibroblasts, as they do in trisomic erythrocytes. Therefore, this finding, which adds yet another mammalian enzyme to the list of enzymes for which the expected gene dosage effect is demonstrable, also serves to confirm the mapping of GARS to chromosome 21.

During the preparation of this manuscript, Scoggin and coworkers (8) published results consistent with those reported here, with dosage effects of 1.54 and 0.47, respectively, in fibroblasts trisomic and monosomic for chromosome 21. They suggested that the increased GARS activity in trisomic cells is a factor contributing to the elevation of the serum uric acid concentration which has been reported in Down syndrome (9). If true, this would explain one of the functional consequences of trisomy 21, and we strongly believe that such functional explanations are important if the mechanisms by which anueploidy produces its deleterious effects are to be understood (10). However, in view of the possibility that enzymes earlier in the pathway may be rate limiting in the de novo purine biosynthetic pathway (11), the hypothesis that increased GARS activity is functionally significant in trisomy 21 remains to be critically tested.

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